Molecular Evolution of the Endemic Genus Tribolodon Species (Teleostei : Cyprinidae) Caused by Paleoenvironmental Changes in the Western North Pacific Region

Molecular Evolution of the Endemic Genus *Tribolodon* Species (Teleostei: Cyprinidae) Caused by Paleoenvironmental Changes in the Western North Pacific Region

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Tribolodon genus is an endemic of Far Eastern seas. At present, there are four species of Far Eastern Daces described, three of them: *Tribolodon hakonensis* (Gunther), *T. brandti* (Dybowski), and *T. ezoe* (Okada et Ikeda) are known for a long time and are common in ichthyofauna of the Sea of Japan, south of the Sea of Okhotsk, and eastern coast of Japanese Islands. The fourth species, *T. nakamurai* (Doi et Shinzawa), the endemic of some rivers of the western coast of Honshu Island, was described only in 2000 (Doi and Shinzawa, 2000). *T. hakonensis* has both anadromous and freshwater types; *T. brandti* has an anadromous life history, whereas *T. ezoe* and probably *T. nakamurai* are river-resident (Sakai et al., 2002). Daces play an important commercial role in the fisheries and aquaculture of the Primorsky region, Sakhalin, and Japan. *Tribolodon* species are the only group of cyprinid fishes, which has anadromous ecotypes adapted to the ocean salinity and forms stable river-resident ecotypes within its natural habitat. Therefore, their biology is interesting and attractive for investigations from the points of view of both basic and applied sciences.

Flora and fauna development cannot take place without close connection with the development of the earth's surface and its environment determining the organic world development. The aim of the present work was to estimate the level of genetic differentiation and the time of divergence among these species on the basis of comparative mitochondrial DNA (mtDNA) analysis in order to restore the past evolutionary and geological events and their interconnection. MtDNA is very suitable for such investigations owing to the rapid rate of its evolution compared to nuclear genes, the absence of recombination, maternal inheritance, and a possibility to estimate the time of taxa divergence with "molecular clock".

Using restriction fragment length polymorphism (RFLP) analysis of mtDNA segments amplified in polymerase chain reaction (PCR), genetic divergence of three species of the genus *Tribolodon: T. hakonensis*, *T. brandti*, and *T. ezoe*, inhabiting rivers and estuaries of the Sea of Japan and the Sea of Okhotsk was studied. Four PCR-products coding five subunits of nadoxide dehydrogenase (ND3/ND4L/ND4/ND5/ND6), subunits 6 and 8 of ATP-synthetase (ATP6/8), subunit of cytochrome oxidase III (COIII), and two big ribosomal RNA (12S/16S) were amplified and analyzed with the set of 13 restriction endonucleases: AvaI, BsuRI, Cfr13I, Hin6I, HindIII, HinfI, MboI, MvaI, PstI, RsaI, StyI, and VspI. *Leuciscus waleckii*, the representative of the common with *Tribolodon* subfamily Leuciscinae was used as an outgroup root.

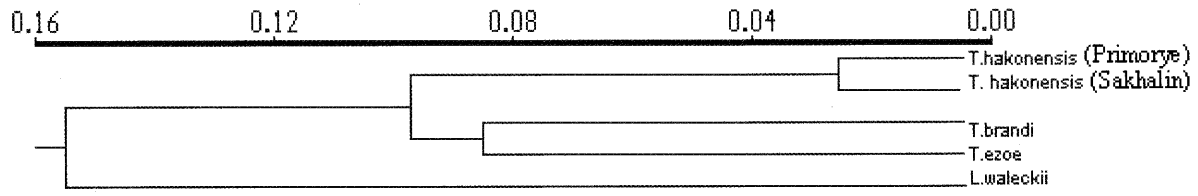

Figure 1 UPGMA-phenogram illustrating the genetic relationships among species of *Tribolodon* genus and *L. waleckii*

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UPGMA-phenogram based on the matrix of genetic distances and illustrating the genetic relationships among species by their mtDNA is presented in the figure. As it seen, haplotypes form two clusters, one of them belongs to *L. waleckii*, another combines all the dace species. The dace cluster divides into two branches, one of which combines two samples of *T. hakonensis*, another one contains *T. brandti* and *T. ezoe*.

Quantitative distinctions among mtDNA nucleotide sequences of all the dace species and *L. waleckii* average out 15%. Distinctions between haplotypes of *T. hakonensis* and the two other dace species *T. brandti* and *T. ezoe* were estimated about 10.9%, between *T. brandti* and *T. ezoe* – about 8.5%. Reasoning from the assumption that speed of nucleotide substitutions in mtDNA of vertebrates is approximately 2% per one million years (Avise, 2000), the time of the independent divergence of *T. hakonensis*, *T. brandti*, and *T. ezoe* was 4-6 million years ago. Thus, the time of the beginning of the divergence corresponds to the border between Miocene and Pliocene. That time was characterized by a number of transgressions and regressions causing such factors of evolution as geographic isolation and salination/desalination of the lake that existed instead of the Sea of Japan. The origin of the genus *Tribolodon* and its salinity tolerance are ascribed exactly back to the Miocene Sea-of-Japan Lake and the Pliocene Paleo-Sea-of-Japan, respectively, important factor of distribution and divergence of these fishes being periodic fluctuation of the ocean level (Lindberg, 1972; Sakai et al., 2002).

On the coasts of Sakhalin Island and Khabarovsky region a form of dace was found that is morphologically similar to *T. hakonensis* of the southern Sea of Japan, but essentially differs from it by the mtDNA (2, 5% of nucleotide substitutions). The level of nucleotide variability of the Sakhalin type of dace (0.011418) is practically by an order of magnitude higher than that of Primorye type (0.001523). The absence of common haplotypes between these two types of *T. hakonensis* suggests the existence of genetic isolation between them during many generations. The time of their divergence (more than 1 million years ago) covers the period between Pliocene and Pleistocene, the time when only freshwater faunas of river systems like Paleo-Amur or Paleo-Shuifen could survive during the transgression of the ocean level, which could serve as a diversifying factor. Since Paleo-Amur was a more extensive river system than Paleo-Shuifen, it can be supposed that its type of *T. hakonensis* successfully survived, adapted to the ocean salinity, and spread along Sakhalin, Khabarovsky region coasts, and presumably, along Japanese coast. Freshwater fish fauna of less extensive Paleo-Shuifen river system became mostly extinct under the transgression influence. So, the low level of Primorye type of *T. hakonensis* genetic variability can be explained by the fact that the Paleo-Shuifen population of *T. hakonensis* might have passed through the bottleneck during repeating inter-glacial and glacial epochs. Thus, the genetic characteristics of *T. hakonensis* from Primorye and *T. hakonensis* from Sakhalin and Japan waters suggest that these two forms may be different species.

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